IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Patent Application of	Conf. No.: 1945
WOLLMER *ET AL.*	Atty. Ref.: 613-101
Serial No. 10/563,828	T.C./A.U.: 1618
Filed: May 8, 2006	Examiner: SAMALA, J. R.

For: MICROEMULSIONS AND ITS USE FOR PREVENTING AIRWAY DISEASES

* *

October 28, 2011

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPEAL BRIEF

Sir:

Appellants submit this Brief to appeal the Examiner's final rejections as set forth in the Office Action mailed September 28, 2010 (the "final Office Action"). The required fee in accordance with 37 CFR § 41.20(b)(2) is submitted herewith.

Since the Notice of Appeal was filed on March 28, 2011, the Brief was initially due on May 28, 2011. Appellants petition for a 5-month extension in the period for response and attach the required fee in accordance with 37 CFR § 1.136. Therefore, this Brief is timely filed.

Reversal of the Examiner's rejections of claims 19-27 by the Board of Patent Appeals and Interferences (the "Board") is respectfully requested.

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(I) REAL PARTY IN INTEREST

The assignee, NARES AB holds all rights in the subject invention, as well as the invention disclosed and claimed therein, by the assignment recorded on May 8, 2006 in the Patent and Trademark Office starting at reel 017911 and frame 0005.

(II) RELATED APPEALS AND INTERFERENCES

Appellants, the undersigned, and the assignee are not aware of any related appeals, interferences, or judicial proceedings (past or present), which will directly affect or be directly affected by or have a bearing on the Board's decision in this appeal.

(III) STATUS OF CLAIMS

Claims 1-18 were previously canceled.

Claims 19-27 have been rejected.

Claims 28-33 were previously withdrawn.

No claims have been allowed.

(IV) STATUS OF AMENDMENTS

No amendments have been filed since the mailing date of the Final Office Action.

(V) SUMMARY OF CLAIMED SUBJECT MATTER

The invention relates to a reversed phase microemulsion comprising 5 to 35 wt% of a non-polar animal or vegetable oil, 10 to 55 wt% of at least one polar solvent selected from the group consisting of water, a buffer, an alcohol, and mixtures thereof, and at least one surfactant selected from a polysorbate, a poloxamer and a fatty acid polyoxyethylene, wherein the microemulsion further comprises 20-50 wt% of a monoacyl glycerol. See claim 19. It is supported explicitly and implicitly throughout the Specification as filed, such as, for example, on page 9, lines 1-3 and 22-31; page 10, lines 13-22; and page 11, 3-14. It is also supported by the subject matter of original claims 1-3, 8-10, 14 and 17.

Alternatively, the invention relates to a reversed phase microemulsion suitable for entrapping airborne particles, consisting of 5 to 35 wt% of a non-polar animal or vegetable oil, 10 to 55 wt% of at least one polar solvent selected from the group consisting of water, a buffer, an alcohol, and mixtures thereof, and at least one surfactant selected from the group consisting of a polysorbate, a poloxamer and a fatty acid polyoxyethylene, wherein the microemulsion further comprises 20-50 wt% of a monoacyl glycerol. See claim 20. It is supported explicitly and implicitly throughout the Specification as filed, such as, for example, on page 9, lines 1-3 and 22-31; page 10, lines 13-22; and page 11, 3-14. It is also supported by the subject matter of original claims 1-3, 8-10, 14 and 17.

Therefore, the invention as presently claimed is clearly supported by Appellants' disclosure as originally filed.

(VI) GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

A. Under 35 U.S.C. 112, 1st paragraph, was it proper to reject claims 19-27 as allegedly lacking written description in the specification?

B. Under 35 U.S.C. 103(a), was it proper to reject claims 19-27 as allegedly unpatentable over Linn *et al.* (U.S. Patent 4,797,273) in view of Rudnic *et al.* (U.S. Patent 5,897,876) and Cho *et al.* (U.S. Patent 5,665,700)?

(VII) ARGUMENT

A. Under 35 U.S.C. 112, 1st paragraph, was it proper to reject claims 19-27 as allegedly lacking written description in the specification?

The specification must convey with reasonable clarity to persons skilled in the art that applicant was in possession of the claimed invention as of the filing date sought. See *Vas-Cath v. Mahurkar*, 19 USPQ2d 1111, 1117 (Fed. Cir. 1991). The description includes "words, structures, figures, diagrams, formulas, etc., that fully set forth the claimed invention." *Lockwood v. American Airlines*, 41 USPQ2d 1961, 1966 (Fed. Cir. 1997). But the Patent Office has the initial burden of presenting evidence or a reason why persons of ordinary skill in the art would not have recognized such a description of the claimed invention in the original disclosure. See *In re Gosteli*, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989).

A device disclosed in the specification that inherently performs a function or has a property, operates according to a theory, or has an advantage necessarily discloses that function, theory, or advantage even though the specification says nothing explicit about the characteristic. See *In re Smythe*, 178 USPQ 279, 285 (C.C.P.A. 1973). An amendment introducing an inherent characteristic of such a device into the claims is not prohibited by the written description requirement. See *id.*

In the Office Action dated September 28, 2010 the Examiner maintained her objection that there is no basis in the application as filed for the term "reversed phase microemulsion" in claims 19-27.

In the previous Office Action, mail dated January 15, 2010, the Examiner stated that the Declaration by the joint inventor Thomas Landh (Evidence Appendix, Exhibit A, signed October 26, 2009 and submitted to the Examiner on October 27, 2009) was not persuasive because it does not mention that the claimed invention is a reversed phase microemulsion. The Examiner also alleged that the prior art teaches an emulsion having a polar phase mixed with non-polar lipids and surfactants which would lead to a reversed phase emulsion. The Examiner objected that the declaration from inventor Landh does not recite the components, amounts or conditions used, so

that she cannot tell whether the compositions of the prior art are the same as the claimed invention.

In response to these objections, Appellants drew to the Examiner's attention that paragraph 12 of inventor Landh's declaration (Evidence Appendix, Exhibit A of this Brief) refers to the "reversed phase microemulsions of the type recited in the claims" of this application. This is clearly a statement that the instant inventions are reversed phase microemulsions.

Moreover, this point is explicitly covered in the declaration by inventor Per Wollmer, dated August 18 2008 (Evidence Appendix, Exhibit B, submitted to the Examiner with the filing of an RCE and Amendment on August 20, 2008). Paragraph 3 states that "the microemulsions of the present invention are "reversed phase" as opposed to "normal" type". The second sentence of paragraph 8 states, "All examples are necessarily reversed phase water-in-oil type microemulsions as is evident to one skilled in the art." Furthermore, the declaration of Per Wollmer clearly stated the logic of these statements. It is clear, therefore, that the declarations of both inventors do, in fact, state that the instant invention is a reversed phase microemulsion. A device disclosed in the specification that inherently performs a function or has a property, operates according to a theory, or has an advantage necessarily discloses that function, theory, or advantage even though the specification says nothing explicit about the characteristic. See *In re Smythe*, 178 USPQ 279, 285 (C.C.P.A. 1973). Appellants have therefore clearly stated the support for the pending claims in compliance with the Regulations.

Nonetheless, in the Office Action dated September 28, 2010 the Examiner stated that Appellants' evidence and statements are not persuasive because:

(1) the statement made in the declaration cannot be a supportive evidence of the originally filed specification,

(2) the declaration does not mention that the claimed invention is a reversed phase microemulsion, and

(3) a general statement that the microemulsions of the current invention are reversed phase, because they could not be anything other than reversed phase, does not mean that the instant invention is related to reversed phase microemulsions.

Appellants submit that it is extremely difficult to construe that the explicit statements from the Inventors that the microemulsions of the current invention are reversed phase to mean anything else. It is especially difficult to construe the explicit statements to not mean that the invention relates to reversed phase microemulsions. Equally difficult is understanding how it can be in doubt that the invention relates to reversed phase microemulsions, given that the microemulsions of the invention cannot be anything other than reversed phase.

This point is crucial to the Examiner's maintained objection that the phrase "reversed phase" has no basis in the application as filed and therefore constitutes addition of matter. As explained in our previous responses and in declarations by two of the joint inventors, the compositions of the invention are inherently reversed phase and this is implicitly and inherently disclosed throughout the application as filed. The reversed phase nature of the microemulsions of the current invention is an intrinsic property, which would be clear to the skilled worker in the field, and the stipulation of this property in the claims therefore has basis throughout the application as filed. It is no extension of the scope of the application as originally filed to specify that the microemulsions of the current invention are reversed phase, because they could not be anything other than reversed phase. The reversed phase nature of the microemulsions of the invention would be immediately and abundantly clear to the skilled worker.

Appellants further note that the Examiner still has not commented on the supporting publication by Leser *et al.* in this regard (Evidence Appendix, Exhibit C, Leser *et al.*, "Self-Assembly of Polar Food Lipids", Advances in Colloid and Interface Science, 123-126 (2006) 125-136, submitted to the USPTO on August 20, 2008 with the filing of and Amendment and an RCE), although it has been drawn to her attention at least twice already in the Responses filed July 9, 2010 and October 27, 2009 and submitted in an IDS on August 20, 2008. Although the Examiner indicated that the IDS submitted on August 20, 2008 was in compliance with the

provisions of 37 C.F.R. 1.97, the Examiner still have not commented on Leser *et al.* and stated why it is not further evidence supporting Appellants' points.

The lipids in the formulations, glycerol monooleate and sesame oil, cannot in themselves form the curvature required for an oil-in-water microemulsion. In other words, the curvature of the lipids used is such that they will inevitably form reversed phase microemulsions unless used with other components having positive curvature in amounts that would dominate the phase behavior. No such dominant components are taught towards in Appellants' Specification. It should thus be clear to one skilled in the art that the invention applies to reversed-phase microemulsions. The article by Leser *et al.* (Evidence Appendix, Exhibit C) contains some discussion on similar systems which supports and is consistent with Appellants' arguments. See, for example, section 3.1.

Appellants urge the Board to reverse the Section 112 rejection because the specification conveys to a person skilled in the art that they were in possession of the claimed invention.

B. Under 35 U.S.C. 103(a), was it proper to reject claims 19-27 as allegedly unpatentable over Linn *et al.* (U.S. Patent 4,797,273) in view of Rudnic *et al.* (U.S. Patent 5,897,876) and Cho *et al.* (U.S. Patent 5,665,700)?

A claimed invention is unpatentable if the differences between it and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art. *In re Kahn*, 78 USPQ2d 1329, 1334 (Fed. Cir. 2006) citing *Graham v. John Deere*, 148 USPQ 459 (1966). The *Graham* analysis needs to be made explicitly. *KSR Int'l v. Teleflex*, 82 USPQ2d 1385, 1396 (2007). It requires findings of fact and a rational basis for combining the prior art disclosures to produce the claimed invention. See id. ("Often, it will be necessary for a court to look to interrelated teachings of multiple patents . . . and the background know-ledge possessed by a person having ordinary skill in the art, all in order to determine whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue"). The use of hindsight reasoning is impermissible. See id.

at 1397 ("A factfinder should be aware, of course, of the distortion caused by hind-sight bias and must be cautious of arguments reliant upon ex post reasoning"). Thus, a prima facie case of obviousness requires "some rationale, articulation, or reasoned basis to explain why the conclusion of obviousness is correct." *Kahn* at 1335; see *KSR* at 1396. Finally, a determination of prima facie obviousness requires a reasonable expectation of success. See *In re Rinehart*, 189 USPQ 143, 148 (C.C.P.A. 1976).

Appellants note that claims 19-27 relate to their work with certain (reversed phase) microemulsions in entrapping airborne particles. In particular, they have surprisingly established that the claimed compositions form "non-breaking" layers at a body surface that serve to entrap airborne particles and thus prevent conditions, such as allergies, which may be caused by these particles.

In contrast to the claimed subject matter, Linn *et al.* relates to skin moisturizing microemulsions. These are water-in-oil microemulsions comprising 1-8 wt.% sunscreen, 15-79 wt.% of microemulsion-forming surfactant, 15-79 wt.% polysiloxane and 5-50 wt.% skin humectant (Linn *et al.*, column 2, lines 14-20) and are designed for complete absorption into the skin.

Several specific microemulsions are disclosed in columns 3-4 of Linn, one of which comprises 3-35 wt.% vegetable oil, 20-80 wt.% microemulsion-forming surfactant, 10-70 wt.% polysiloxane and 5-50 wt.% skin humectant.

In contrast, the reversed phase microemulsion of Appellants' claimed invention requires:

> 5-35 wt.% of a non-polar animal or vegetable oil;
>
> 10-55 wt.% of a polar solvent selected from water, buffer, alcohol or mixtures thereof;
>
> at least one surfactant from polysorbate, poloxamer or fatty acid polyoxyethylene;

and 20-50 wt.% monoacyl glycerol.

There is no suggestion in Linn that the microemulsions could be formed without the major polysiloxane or skin humectants components, and in particular nothing to suggest a monoacylglycerol, a non-polar vegetable oil and a surfactant could be used in combination without the other key components. This is emphasized by the fact that monoacylglycerols and poloxamers must be selected from very long lists and non-polar vegetable oils are not disclosed by Linn.

Linn *et al.* discloses various vegetable oils (Lynn *et al.*, column 5, lines 46-50) but does not teach that these must be non-polar. A very long list of surfactants is disclosed for use by Linn *et al.* (column 6, lines 14-34), including sorbitols and poloxamers. However, there is no teaching that these are particularly advantageous or any incentive for their selection from the list.

In addition, there is no disclosure of the incorporation of a monoacyl glycerol into the microemulsions of Linn *et al.* This is the component of the present claims having the largest minimum level. It is also a component which has a key role in determining the structure and phase-behavior of the present systems.

Linn *et al.* teaches compositions which "leave little residue on the surface of the skin" and are not irritants when the correct balance of surfactants is used (Linn *et al.*, column 7, lines 51-56). Linn *et al.* teaches that their compositions are particularly important because of their ability to increase the rate of penetration of the moisturizing agents incorporated to the areas of skin in need of moisturizsation, i.e., the dermis and epidermis (Linn *et al.*, column 8, lines 20-31), their ability to increase epidermal thickening (Linn *et al.*, column 8, lines 31-35), and their ability to increase the penetration of a macroemulsion moisturizer subsequently applied (Linn *et al.*, column 8, lines 36-52).

For at least the above reasons, claims 19-27 are not obvious in view of Linn *et al.* The addition of Rudnic *et al.*, does not cure the many defects of Linn *et al.*

Rudnic *et al.* teaches a water-in-oil microemulsion made up of an oily phase, an aqueous phase and a surfactant. The oily phase is disclosed as made up of long chain fatty acids or esters/alcohols thereof. An extremely large number of possible compounds are listed in column 3, line 55 to column 4, line 59 (Rudnic *et al.*). Many suitable surfactants are listed in column 5, lines 10-44 (Rudnic *et al.*). The aqueous phase is simply disclosed as being "mainly" water (Rudnic *et al.* column 3, lines 51-52).

Rudnic *et al.* does not disclose the necessary components of the current invention, or their relative proportions. In particular, the incorporation of 20-50 wt.% monoacyl glycerol is not disclosed or rendered obvious therein. Appellants emphasize again that this is a key component and is required in the presently claimed invention and which is not disclosed or rendered obvious in view of Rudnic *et al.*

The pharmaceutical formulations of Rudnic *et al.* are for oral delivery (see abstract). In contrast, Linn *et al.* relates to a topical product for sub-dermal effect. There is thus no reason for the skilled worker to combine these documents. There is no incentive provided by Rudnic *et al* for the skilled man to modify the teachings of Linn *et al.* and, crucially, the necessary teaching required to reach the current microemulsions from the microemulsions of Linn *et al.* is not supplied by Rudnic *et al.* A skilled worker would not look to Rudnic if making dermal compositions, nor to Linn if making oral compositions. Even if both were consulted, however, there is no suggestion that reversed phase microemulsions are formable from the very specific components and proportions recited in the present claims. Thus, there is no reasonable expectation of success even if the two references were combined. Here, the Examiner has not established a prima facie case of obviousness because no evidence of a reasonable expectation of success has been provided. To reach the claimed result, the skilled worker must try thousands of combinations from two unrelated documents and then optimize each for a function not proposed in either document. This is simply not a viable task.

The addition of Cho *et al.* does not cure the defects of Linn *et al.* and Rudnic *et al.* Cho *et al.* relates to oral administration of pharmaceutical compositions, and fails to disclose or render obvious the necessary modifications to the microemulsions of Linn *et al.*

The microemulsions of Linn *et al.* are ultimately effective at the sub-dermal level, rather than on the surface of the skin. In fact they are not intended to leave a residue on the surface and it is taught that the lack of surface residue is an advantage of these compositions.

In contrast, the microemulsions of the current invention are intended to coat the surface of a mucosal membrane, in order to substantially enclose and "trap" airborne particles (see paragraphs [0026] to [0030] of Appellants' Specification). The problem to be solved by Appellants' claimed subject matter may be defined as the prevention of airway diseases and/or inflammation of mucosal membranes. The solution to this problem is the microemulsion of claims 19-27 which remain on the surface without "breaking".

When seeking to solve the problem of the prevention of airway diseases, one of ordinary skill in the art would not consider documents regarding oral administration of pharmaceuticals, such as Rudnic *et al.* and Cho *et al.* Neither would he consult teachings regarding the application of cosmetics such as moisturizer or sun-screen at a sub-dermal level, as in Linn *et al.* There is absolutely no reason or motivation for one of ordinary skill in the art to even consult, let alone combine these documents.

In response to Appellants' previous Amendments and Responses related to the points stated above, the Examiner alleged, in the Office Action dated September 28, 2010, that because current claim 19 is in "comprising" language it does not exclude the presence of polysiloxane and humectants, and that Linn *et al.* discloses a water-in-oil microemulsion comprising 3-35 wt.% vegetable oil and 20-80 wt.% of microemulsion-forming surfactant which reads onto alcohol. The Examiner alleged that Linn *et al.* teaches sesame oil, which is a non-polar vegetable oil, and that the lack of teaching in respect of any advantage or incentive for choosing this particular type of vegetable oil, i.e. non-polar oil, is not significant. The Examiner further alleged that the lack of teaching with regard to any incentive or advantage that could motivate the skilled worker to select the necessary surfactants from the very long lists disclosed by Linn *et al.* does not matter because the skilled worker would be motivated to use all the disclosed surfactants for microemulsions with a reasonable expectation of success.

Appellants note, as mentioned above, the reversed phase microemulsion of the current invention requires:

5-35 wt.% of a non-polar animal or vegetable oil;

10-55 wt.% of a polar solvent selected from water, buffer, alcohol or mixtures thereof;

at least one surfactant from polysorbate, poloxamer or fatty acid polyoxyethylene;

and 20-50 wt.% monoacyl glycerol.

The Examiner dismissed Appellants' position that there is no suggestion in Linn that a monoacylglycerol, a non-polar vegetable oil and a surfactant could be used in combination without the other key components. The Examiner stated that Linn *et al.* is relied upon to show that it is known in the art to make water-in-oil microemulsions comprising vegetable oil, surfactant and polyhydric alcohols such as propylene glycol, glycerin or sorbitol to provide superior results when applied to skin following their application. Rudnic *et al.* is relied upon to teach that water-in-oil microemulsions can contain long chain fatty acids or esters such as glycerol monooleate. Rudnic *et al.* and Cho *et al.* are relied upon to teach knowledge in the art of employing mono-acyl glycerides such as glycerol monooleate into microemulsions and the Examiner stated that this function would remain the same regardless of whether the microemulsions were administered topically or orally.

Appellants respectfully submit that the Examiner has failed to establish a prima facie case of obviousness because a vital point is missing. The claimed compositions form layers at a body surface. The Declaration by inventor Wollmer pointed out that the current compositions can form barrier layers. This behavior is the key to the reduction in intensity of allergic reactions demonstrated in example 5 of Appellants' Specification.

The compositions of Linn *et al.* are absorbed into the skin - they "leave little residue on the surface of the skin" (Linn *et al.*, column 7, lines 51-56) and increase the rate of penetration of the moisturizing agents incorporated to the areas of skin in need of moisturisation, i.e. the dermis and epidermis (Linn *et al.*, column 8, lines 20-31), they increase epidermal thickening (Linn *et*

al., column 8, lines 31-35), and they increase the penetration of a macroemulsion moisturizer subsequently applied (Linn *et al.*, column 8, lines 36-52).

In other words, the compositions of Appellants' claimed invention have properties which are the opposite of the compositions of Linn *et al.*

The Declaration by inventor Landh confirms that a minimum of 20 wt.% glycerol monooleate is necessary in order to form reversed phase microemulsions of the type necessary in the instant invention, i.e. necessary to achieve barrier layer behavior. The compositions of Linn, Rudnic and Cho, individually or in combination, do not contain the necessary minimum amount of glycerol monooleate required to achieve the layer formation of claims 19-27.

The Examiner contended that it requires simple optimization of the compositions of Linn *et al.* to obtain the current compositions, but in this case it would be necessary both to import into Linn a key component, monoacyl glycerol, and to "optimize" its content beyond what is disclosed by Rudnic and Cho, and with what aim? The compositions of Linn *et al.* are penetrative moisturizers. Modification as the Examiner envisions would not lead to improvement in respect of this behavior but deterioration, since the currently claimed compositions have poor penetration of the skin - they block penetration by forming a barrier layer. Why then, would the skilled worker consider making such a change? There is nothing in the cited documents to recommend it and there is no expectation of success since the compositions of Linn do the reverse.

The Examiner stated that Rudnic and Cho teach knowledge in the art of employing mono-acyl glycerides such as glycerol monooleate into microemulsions and that this function would remain the same regardless of whether the microemulsions were administered topically or orally. However, the only reason for incorporating glycerol monooleate into the compositions of Linn *et al.* would be to improve or at least equal the properties of penetrative moisturizing taught by Linn *et al.*, unless there was some alternative teaching in any of Linn, Rudnic or Cho that other effects were possible and/or advantageous. There is no teaching in Rudnic, Cho or their combination that glycerol monooleate has an effect on this behavior, either for better or worse.

Hence there is no motivation for making this change, and no reason for the skilled worker to develop the claimed compositions on the basis of the cited documents.

Appellants submit that the skilled worker, seeking to solve the problem of the prevention of airway diseases, would not consider documents regarding oral administration of pharmaceuticals, such as Rudnic *et al.* and Cho *et al.* Nor would the skilled worker, considering this problem, have any reason to consult teachings regarding the application of cosmetics such as moisturizer or sun-screen at a sub-dermal level, as in Linn *et al.* There is absolutely no reason why the skilled worker would even consult, let alone combine these documents, except with hindsight knowledge of the current invention.

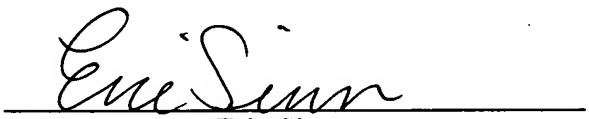
For the reasons given above, it is believed that claims 19-27 are inventive over the combination of the cited art. Appellants urge the Board to reverse the Section 103 rejection because their claimed invention would not have been obvious to one of ordinary skill in the art.

CONCLUSION

In conclusion it is believed that the application is in clear condition for allowance; therefore, early reversal of the Final Rejection and passage of the subject application to issue are earnestly solicited.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: 

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(VIII) CLAIMS APPENDIX

1.-18. (Canceled).

19. (Previously Presented) A reversed phase microemulsion comprising 5 to 35 wt% of a non-polar animal or vegetable oil, 10 to 55 wt% of at least one polar solvent selected from the group consisting of water, a buffer, an alcohol, and mixtures thereof, and at least one surfactant selected from a polysorbate, a poloxamer and a fatty acid polyoxyethylene, wherein the microemulsion further comprises 20-50 wt% of a monoacyl glycerol.

20. (Previously Presented) A reversed phase microemulsion suitable for entrapping airborne particles, consisting of 5 to 35 wt% of a non-polar animal or vegetable oil, 10 to 55 wt% of at least one polar solvent selected from the group consisting of water, a buffer, an alcohol, and mixtures thereof, and at least one surfactant selected from the group consisting of a polysorbate, a poloxamer and a fatty acid polyoxyethylene, wherein the microemulsion further comprises 20-50 wt% of a monoacyl glycerol.

21. (Previously Presented) The microemulsion as claimed in claim 19 or claim 20 wherein said mono-acyl glyceride is glyceryl monooleate, glyceryl monolinoleate or glyceryl monolinenoleate.

22. (Previously Presented) The microemulsion as claimed in claim 19 or 20, wherein said non-polar animal or vegetable oil comprises sesame oil.

23. (Previously Presented) The microemulsion as claimed in claim 19 or 20, wherein at least one component of said polar solvent has a pH exceeding pH 5.5.

24. (Previously Presented) The microemulsion as claimed in claim 19 or 20, wherein said polar solvent comprises propylene glycol and/or polyethylene glycol and/or saline solution.

25. (Previously Presented) The microemulsion as claimed in claim 19 or 20, wherein said surfactant has a hydrophilic-hydrophobic balance exceeding 7.

26. (Previously Presented) The microemulsion as claimed in claim 19 or 20 wherein said polysorbate is polysorbate 80.

27. (Previously Presented) A composition suitable for administration to peripheral membrane linings of the nose, the eyes, the ears, the pharynx, and/or the larynx of a mammal, the composition comprising a pharmaceutically effective amount of a microemulsion as claimed in claim 19 or 20.

28. (Withdrawn) A mouth or nasal spray device containing the microemulsion as claimed in claim 19 or 20.

29. (Withdrawn) A filter device comprising the microemulsion as claimed in claim 19 or 20.

30. (Withdrawn) A mouth or nasal spray device containing the composition as claimed in claim 27.

31. (Withdrawn) A method for trapping airborne particles directly or indirectly causing allergic rhinitis in a subject, said method comprising contacting at least one surface of said subject with a composition as claimed in claim 26.

32. (Withdrawn) A method of trapping airborne particles directly or indirectly reaching exterior mucosal membranes of a mammal, said method comprising the step of administering to said exterior mucosal membranes of said mammal a prophylactically effective amount of a composition as claimed claim 27.

33. (Withdrawn) The method as claimed in claim 32, wherein said composition is administered buccally or intranasally.

(IX) EVIDENCE APPENDIX

A. Evidentiary Declaration of inventor Thomas Landh submitted to the USPTO on October 27, 2009.

B. Evidentiary Declaration of inventor Per Wollmer submitted to the USPTO on August 20, 2008.

C. Leser *et al.*, "Self-Assembly of Polar Food Lipids", Advances in Colloid and Interface Science, 123-126 (2006) 125-136.

A. Evidentiary Declaration of inventor Thomas Landh submitted to the USPTO on October 27, 2009.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

WOLLMER ET AL.	Atty. Ref.: 613-101; Confirmation No. 1945
Appl. No. 10/563,828	TC/A.U. 1618
Filed: May 8, 2006	Examiner: Samala

For: MICROEMULSIONS AND ITS USE FOR PREVENTING AIRWAY DISEASES

* * * * * * * * * * * *

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

EVIDENTIARY DECLARATION UNDER 37 CFR §1.132

I, Tomas Landh, one of the joint inventors in the above-identified application, do hereby declare:

1. That my residence and citizenship are of record in this application as stated in my declaration as inventor made under 37 C.F.R.§1.63 and that I am employed by Novo Nordisk A/S, Denmark.

2. That I am familiar with the contents of the above-identified application and the research effort underlying this application.

3. That I have read and am familiar with the Office Action of 28 April 2009 and with the contents of US 6506803 and US 6618840.

4. That according to traditional emulsion technology an "emulsion" is formed of discrete droplets dispersed and kinetically stabilised in a continuous phase. In an "oil in water" emulsion, stabilised droplets are dispersed in an aqueous environment.

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5. That traditional oil in water emulsions are cloudy in appearance and are discontinuous with respect to the oil phase i.e. it does have a continuous structure and are thermodynamically unstable.

6. That in contrast, to "emulsions", "microemulsions", such as the microemulsions of the present invention, are based on mixtures of polar and non-polar lipids which typically form transparent, thermodynamically stable more or less structured phases.

7. That the phase structures available to pure lipids depend upon their chemical structure and resultant intrinsic properties, such as spontaneous curvature.

8. That the phase structures available to a mixture of amphiphilic components depends upon the properties of the individual components, upon their ratios and upon their interactions. Thus a component with negative curvature may be present in a mixture exhibiting positive curvature as a whole.

9. That the phase structure of a microemulsion is a property of the composition as a whole and is dependent upon many factors including the amount of each component having positive or negative spontaneous curvature, the degree of that curvature in each case, and the interactions of the components..

10. That I have studied compositions of the type recited in the claims of the above-identified application and have observed their properties and phase behaviour.

11. That I have observed the need for at least a minimum amount of glycerol monooleate in compositions of the type claimed in the above-identified application in order to form reversed phase microemulsions.

12. That based upon my practical experience of microemulsions I believe that it is necessary to have at least around 20 % glycerol monooleate in order to form the reversed phase microemulsions of the type recited in the claims of the above-identified application.

13. That based upon my practical experience of working with microemulsions, I believe that the combinations of components indicated in US 5618840 and US 6596803 would lead to the formation of traditional emulsions rather than reversed phase microemulsions

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I declare further that all statements made herein of my/our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 26 October 2009

Tomas Landh

B. Evidentiary Declaration of inventor Per Wollmer submitted to the USPTO on August 20, 2008.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

WOLLMER ET AL.	Atty. Ref.: 613-101; Confirmation No. 1945
Appl. No. 10/563,828	TC/A.U. 1618
Filed: May 8, 2006	Examiner: Samala

For: MICROEMULSIONS AND ITS USE FOR PREVENTING AIRWAY DISEASES

* * * * * * * * * * * *

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

EVIDENTIARY DECLARATION UNDER 37 CFR §1.132

I, Per Wollmer, one of the joint inventors in the above-identified application, do hereby declare:

1. That my residence and citizenship are of record in this application as stated in my declaration as inventor made under 37 C.F.R.§1.63 and that I am employed by Nares AB of Akarp, Sweden, the assignee of the application.

2. That I am familiar with the contents of the above-identified application and the research effort underlying this application, and that I have read and am familiar with the Official Action of February 21, 2008.

3. That the microemulsions of the present invention are "reversed phase" as opposed to "normal" type.

4. That the phase structures available to lipids depend upon their chemical structure and resultant intrinsic properties, such as spontaneous curvature.

5. That the property of spontaneous curvature is used in the art to predict certain properties of a lipid formulation, such as a resultant phase structure.

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6. Lipids with negative spontaneous curvature tend to form "reversed phase" structures.

7. The components used in the compositions of our invention have negative spontaneous curvature.

8. The lipids in the formulations used in the Examples of the subject application (glycerol monooleate and sesame oil), have a negative spontaneous curvature and cannot form the positive curvature required for an oil-in-water microemulsion. All examples are thus necessarily reversed-phase water-in-oil type microemulsions as is evident to one skilled in the art.

9. Based upon my experience with microemulsions I believe that the advantages of reversed phase microemulsions include retention of their structure on mixing with an aqueous solution which conveys the ability to form a barrier layer. An important issue to note in the context of non-obviousness is the definition of the compositions as presently claimed. In particular the solvent content stated is 10 to 55% by weight of the composition and corresponds to the proportion of solvent illustrated in the Examples of the application as filed. This relatively low proportion of solvent will result in *reversed phase* microemulsions rather than *normal phase*.

10. The lipids in the formulations, glycerol monooleate and sesame oil, do not either alone or in combination possess the intrinsic spontaneous curvature required to form normal phase structures. Neither do they have solubilities in aqueous fluids allowing them to form the curvature required to generate phase-separated normal structures. It follows that both phase behaviour and solubility are collective properties of the mixture. Thus the structure applied in the current invention will be maintained in biological fluids for the time periods relevant to the use of this invention, as will be evident to one skilled in the art.

11. In view of the above, it should be clear to one skilled in the art that the invention applies to reversed microemulsions. This is confirmed in the attached articles, "Self-assembly of polar food lipids", M. E. Leser, L. Sagalowicz, M. Michel and H. J. Watzke, Advances in Colloid and Interface Science, Vol. 123 (2006), pp 125-136, and "Monoglyceride self-assembly structures as delivery vehicles", L. Sagalowicz, M. E. Leser, H. J. Watzke and M. Michel, Trends in Food Science and Technology, Vol. 17 (2006), pp. 204-214. These review articles contain discussions of similar systems.

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Date: 18 August 2008

Per Wollmer

C. Leser *et al.*, "Self-Assembly of Polar Food Lipids", Advances in Colloid and Interface Science, 123-126 (2006) 125-136.

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Advances in Colloid and Interface Science 123–126 (2006) 125–136

ADVANCES IN COLLOID AND INTERFACE SCIENCE

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Self-assembly of polar food lipids

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Available online 11 October 2006

Abstract

Polar lipids, such as monoglycerides and phospholipids, are amphiphilic molecules commonly used as processing and stabilization aids in the manufacturing of food products. As all amphiphilic molecules (surfactants, emulsifiers) they show self-assembly phenomena when added into water above a certain concentration (the critical aggregation concentration). The variety of self-assembly structures that can be formed by polar food lipids is as rich as it is for synthetic surfactants: micelles (normal and reverse micelles), microemulsions, and liquid crystalline phases can be formulated using food-grade ingredients.

In the present work we will first discuss microemulsion and liquid crystalline phase formation from ingredients commonly used in food industry. In the last section we will focus on three different potential application fields, namely (i) solubilization of poorly water soluble ingredients, (ii) controlled release, and (iii) chemical reactivity. We will show how the interfacial area present in self-assembly structures can be used for (i) the delivery of functional molecules, (ii) controlling the release of functional molecules, and (iii) modulating the chemical reactivity between reactive molecules, such as aromas.

Contents

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doi:10.1016/j.cis.2006.07.003

1. Introduction

Analyzing the information gathered in Food Science and Engineering so far, we realize that most of our understanding is on the chemistry and functionality of individual food molecules, such as flavor chemistry, oxidation phenomenon, nutritional functionality, etc. on the one hand, and on the bulk behavior of food materials, such as rheological or textural properties, on the other hand. Investigation of structure formation in food materials has been ignored for a long time, since food engineers knew little about the underlying science linking food structure to product properties [1]. However, it becomes more and more evident that the essential properties of food critically depend on phenomena or processes taking place at different length and timescales. Understanding, for instance, self-assembly structure formation will help bridge the gap which exists between Food Chemistry, describing the molecular properties of food molecules, and Food Physics, describing the macroscopic properties of food products [2]. The detailed information available on the main food components must be supplemented with more information on the interaction between the different components and structural entities. The characteristic properties of food systems are more dictated by the interactions between its components than by the properties of the individual components themselves [3].

One important class of components in food is the lipids. Different classes of lipids exist, namely polar lipids (e.g. phospholipids, glycolipids) and nonpolar lipids (e.g. triglycerides, waxes) [4]. Polar lipids usually are referred to as surface active lipids, amphiphiles or low molecular weight emulsifiers (or surfactants) due to the fact that they consist of both a lipophilic and a hydrophilic part [4]. Polar lipids are also abundant in nature, e.g., in living organisms. One class of polar food lipids obtained directly from nature without chemical conversion is the phospholipids. Table 1 summarizes the most commonly used polar lipids in food and their typical applications [5]. Nowadays, the total world production of polar lipids is estimated to be in the order of 300,000 metric tons [5]. This includes approximately 20 different types of emulsifiers. But, monoglycerides and mono-diglycerides, which are glycerol fatty acid esters, and their derivatives account for about 70% of the world production of food polar lipids. They are considered as the most important group of amphiphiles in food [5]. The use of mono-diglycerides dates back to the 1930s when they were first used in margarine production [6]. Their major applications today are in bread, cakes, margarine, ice cream, or chewing gum. Bakery is by far the biggest application; approximately 60% of all monoglycerides are used in this industry [5]. Most of the polar lipids used in food industry are non-ionic or anionic. The phospholipids are the only zwitterionic surfactants. Due to their toxicity, cationic surfactants are not used.

Food emulsifiers are typically used as processing aids for the production and stabilization of emulsions and foams. They can have numerous functional roles in a large variety of products (see Table 1). For instance, phospholipids are used to realize more than 30 different functional roles (Table 2) [7]. Examining these roles in more details, one realizes that most of the functionalities are based on five main physicochemical processes:

(a) adsorption at interfaces or on solids (such as in emulsions or foams),
(b) promotion of wetting phenomena,
(c) co-crystallization,
(d) complex formation (with proteins or starch components)
(e) self-association (or self-assembly structure formation).

2. Self-assembly properties of polar lipids

As all surfactants, polar lipids used in food industry show self-assembly phenomena if their concentration is higher than a certain 'critical aggregation concentration', denoted as cac [8]. 'Self-assembly' stands for the autonomous organization of components into patterns or structures without human intervention. Nanometre-scale molecular self-assembly is governed

Table 1
Polar food lipids (emulsifiers) and some specific characteristics; adapted from [5,9]

	Abbreviation	EEC number	HLB[a]	Typical applications
Phospholipids	PL	E322	6–9	Margarine, chocolate, baked goods, sauces, instant drinks, pasta, fats
Mono-diglycerides	MG	E471	3–6	Margarine, whipped toppings, ice cream, baked goods, pasta
Acetic acid ester of mono-diglycerides	ACETEM	E472a		Fruits, nuts, pizza
Lactic acid ester of mono-diglycerides	LACTEM	E472b		Baked products, whipped toppings
Citric acid ester of mono-diglycerides	CITREM	E472c		Baked goods, spreads, sauces, meat products
Diacetyl tartaric acid ester of MGs	DATEM	E472e		Baked goods, dairy product analogues
Salts of fatty acids (Na, K)	–	E470		
Polyglycerol ester of fatty acids	PGE	E475		Icings, fillings, confectionery, emulsions, cereals
Propylene glycol ester of fatty acids	PGMS	E477		Cake mixes, whipped toppings
Sodium stearoyl lactylate	SSL	E481	40	Bread, coffee whiteners, fat emulsions, starch-based products, cereals
Calcium stearoyl lactylate	CSL	E482		Bread, fat emulsions, cereals
Sucrose ester of fatty acids	–	E473	6–15	Sauces, canned liquid coffee, sausages, surface treatment fresh fruits
Sorbitan monostearate	SMS	E491	4.7	Yeast for baking, confectionery, fats
Polysorbate 60	PS 60	E435	14.9	Modification of fat crystallization, sauces
Polysorbate 65	PS 65	E436	10.5	Modification of fat crystallization, sauces
Polysorbate 80	PS 80	E433	15	Modification of fat crystallization, sauces

[a] Hydrophilic lipophilic balance.

Table 2
Functionalities of phospholipids, adapted from [7]

Adhesion aid	Emulsifier, surfactant	Plasticizer
Antibleed agent	Encapsulating agent	Release agent (antisticking)
Anticorrosive	Flocculant	Spreading agent
Antidusting agent	Grinding aid	Stabilizer
Antioxidant	Lubricant	Strengthening agent
Antispatter agent	Machining aid	Suspending agent
Biodegradable additive	Mixing and softening agent	Synergist
Catalyst	Modifier	Viscosity modifier
Colour intensifier	Moisturizer	Water repellent
Conditioning agent	Nutritional supplement	Wetting agent
Dispersing agent	Penetrating agent	

Table 3
Self-assembly structures formed by polar food lipids

Polar lipid	Temperature °C	Self-assembly structure
Polysorbate 80 (Tween 80)	20	Normal micelles (L_1)
Polysorbate 60 (Tween 60)	45	Normal hexagonal phase (H_1)
Phosphatidylcholine (lecithin)	20	Lamellar liquid crystalline phase (L_α)
Glycerol monostearate (saturated MG)	20	Lamellar crystalline phase (L_c)
Glycerol monooleate (unsaturated MG)	20	Reversed bicontinuous cubic phase (V_2)
Glycerol monolinoleate (unsaturated MG)	60	Reversed hexagonal phase (H_{II})
Glycolipid	20	Reversed micellar cubic phase (I_2)

by a delicate balance of different non-covalent forces exhibited between the molecules, such as electrostatic, or van der Waals forces [8]. In Table 3 some examples of self-assembly structures formed by polar food lipids are presented. Note that food emulsifiers, as synthetic surfactants, form a variety of different self-assembly structures.

In the present review we will concentrate on the discussion of self-assembly phenomena observed with commercially available polar food lipids both in the absence or presence of food grade oils. Note that it is a common practice to use not only one type but two- or three different types of emulsifiers in a product in order to achieve multiple functionalities. Consequently, it is not trivial for Food Colloid Scientists to link lipid self-assembly structure formation to the final product characteristics, since in almost all cases no information on the basic phase behavior properties of the used lipid mixture is available. Ongoing research on food surfactants aims at better understanding the physicochemical properties, especially the self-assembly characteristics, of single polar lipid ingredients (which are already themselves mixtures of different amphiphilic molecules) in order to better describe the correlation between the chemical structure of a lipid molecule and its impact on the final food product properties.

In the next sections we will describe examples of polar lipid self-assembly formation. First, we will discuss the basics of microemulsions made of food-grade ingredients. Then, formation of food-grade mesophase particles and their physical properties will be described. In the last section we will discuss some ideas as to how these self-assembly structures can be utilized in the context of food.

3. Food-grade microemulsions

3.1. Classical food microemulsions

Microemulsions are macroscopically homogeneous mixtures of oil, water, and surfactant, which on the nanoscopic level consist of individual domains of oil and water separated by a monolayer of amphiphile [8]. Their spontaneous formation and thermodynamic stability clearly differentiate them from macroemulsions which are thermodynamically unstable and need energy to form. The spontaneous formation of microemulsions is based on the underlined surfactant self-assembly phenomenon.

These exceptional physical properties make microemulsions an attractive self-assembly system for food applications. This was recognized already more than 30 years ago and investigated by several academic and industrial research labs [10–12]. Fig. 1 shows a phase diagram [13] which is typically obtained when using food-grade ingredients. It is one of the first published diagrams of its kind. Since monoglycerides and triglycerides are ubiquitous in food, they are attractive components for the formulation of food-grade microemulsions. Their phase diagram is dominated by two single-phase regions, namely a w/o microemulsion phase (L_2) and a lamellar liquid crystalline phase (D). The w/o microemulsion is the dominating isotropic phase. No o/w microemulsion is formed in the water corner since the monoglyceride is too lipophilic for the formation of normal-type self-assembly structures (having a positive spontaneous curvature). Note that the L_2 region is quite small when compared to the extent of the L_2 phase observed in synthetic surfactant–hydrocarbon–water systems, such as the AOT–hydrocarbon oil–water or alkyl ethoxylate–hydrocarbon–water systems [14]. Only small amounts of water can be solubilized into the tricaprylin oil (C_{10} fatty acid chains) in the presence of

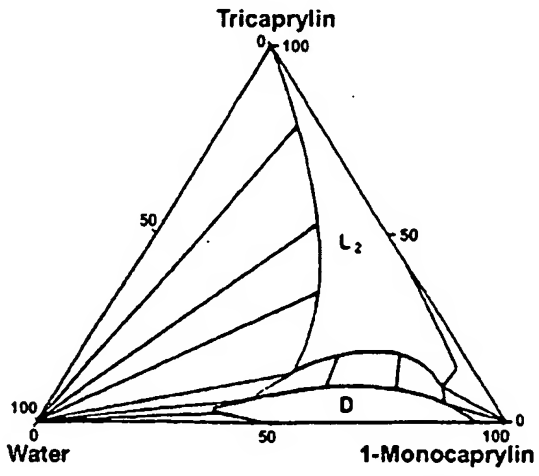

Fig. 1. Typical phase diagram of a polar lipid–water–triglyceride system (reprinted with permission from [13]).

monocaprylin as polar lipid. To reach 15% by weight of water, 50% of emulsifier is needed (see Fig. 1). The water solubilization capacity of the monoglyceride–triglyceride oil mixture is even worse if soybean oil (consists of mainly $C_{18:2}$ chains) is used instead of tricaprylin: in order to obtain an L_2 phase containing 15% water, at least 75% emulsifier is needed. Such a microemulsion does not contain more than 10% soybean oil [15].

A few attempts have been described in the literature to improve the water solubilization capacity in surfactant–vegetable oil systems. One possible way is to add a co-surfactant, such as an alcohol. Leser et al. studied the effect of adding different alcohols to an MCT (medium chain triglyceride; C_8, C_{10} chains)–water (1/1 by weight) mixture in the presence of phosphatidylcholine (PC) as polar lipid [16]. While in the absence of alcohol, the PC is neither well dissolved in the MCT nor in the water (PC is mainly present as 'insoluble' liquid crystals), in the presence of alcohol the liquid crystals are destroyed and isotropic phases are formed. Addition of alcohol seems to decrease the rigidity of the PC bilayers and tunes the PC–alcohol monolayer either towards a negative (w/o type; after addition of *n*-pentanol) or positive (o/w type after addition of *n*-propanol) spontaneous curvature. However, also in these systems, the needed amount of surfactant (i.e., PC) to microemulsify the MCT/water 1:1 mixture is relatively high: in order to obtain an isotropic L_2 phase at least 20% PC and 10% *n*-pentanol are needed. Agterof et al. showed that the alcohols could be replaced by monoglycerides for microemulsification of MCT–water mixtures using PC as the surfactant [17].

Much less systematic research results have been published on the formation of food-grade oil-in-water microemulsions. Early work of Ekman and Lundberg [18] showed that aqueous solutions of egg lecithin or dipalmitoyl phosphatidylcholine could solubilize up to 15% by weight triolein ($C_{18:1}$ chains). As found for triglyceride based w/o microemulsions, the formulation of triglyceride based o/w microemulsions is also not an easy task. In general much less triglyceride oil than mineral oil can be solubilized into o/w microemulsions [19]. Again the use of an alcohol, such as ethanol, increases to a certain extent the oil solubilization capacity of the emulsifier.

3.2. Limitations in the formulation of food microemulsions

Experimental work on triglyceride containing microemulsions has shown that the triglyceride oil containing surfactant–water systems do not follow the classical empirical 'rules' which can be used to predict the phase behavior of synthetic surfactant–water hydrocarbon oil mixtures [20,21]. A different strategy must be employed in order to optimise triglyceride containing microemulsions. The main limitations are the following:

- In food the choice of components which can be used as efficient surfactants is very limited (regulatory issues)
- The amount of surfactant and/or co-surfactant needed to microemulsify a certain water–oil mixture is quite high (much higher than needed for the microemulsification of, for instance, hydrocarbon oils) [16].
- Using triglycerides as oil, it is not possible to find conditions that allow to form a Winsor III (or D-middle phase microemulsion [20,21]) system. This means that it is very difficult to create bicontinuous microemulsions which contain equal amounts of oil and water in the presence of vegetable oils.
- The presence of triglyceride oils does not allow to induce classical phase inversion [20,21] from a Winsor I via a Winsor III into a Winsor II system by increasing, for instance, temperature.
- In most cases, high temperatures are needed to form the microemulsions.

In conclusion, it is speculated that triglyceride molecules are (i) either too large molecules and/or (ii) too amphiphilic for an easy formation of microemulsions. They seem to interact with the surfactant hydrocarbon chains in a different way than the hydrocarbon oils do. Hamilton and Small [22] provided some experimental evidence using NMR. They showed that triglyceride molecules, which are solubilized into phospholipid bilayers, are incorporated with their carbonyl groups (their 'head-group') at the aqueous interface, i.e., are in direct contact with the aqueous phase, whereas hydrocarbon oils are simply dissolved into the hydrocarbon chain region of the phospholipid molecules without interacting with the water molecules. Therefore, the difficulty in forming microemulsions with triglycerides might be connected with their slight amphiphilic character giving them co-surfactant properties.

3.3. Improved solubilization in food microemulsions

These limitations significantly curtail the potential use of microemulsions in the context of food. Therefore, it is necessary to develop new strategies which (i) allow to significantly increase the water and food-grade oil solubilization capacities of w/o and o/w microemulsions, respectively, and (ii) allow to find a w/o microemulsion which transforms continuously into an o/w microemulsion, i.e., without inducing phase separation, upon addition of an aqueous phase. This is an important prerequisite for applications in which, for instance, one tries to add a concentrated microemulsion to an aqueous product, such as a beverage (see Section 5), without inducing inhomogeneity in the product. Most food microemulsion formulations, as for instance the L_2 phase obtained in the Tricaprylin–monocaprylin–water mixture (shown in Fig. 1) cannot be added to water without separating into two or more phases.

One possible strategy to achieve these two goals consists of:

(i) replacing the triglyceride oil with an essential oil, as, for instance, the R(+)-limonene. Since essential oils have a lower molecular weight than triglycerides, and a non-amphiphilic molecular structure, it is expected that microemulsification of such oil–water systems will be easier to accomplish;

(ii) taking a more hydrophilic surfactant which is both oil and water soluble; monoglycerides are too lipophilic (HLB is around 3), and are almost insoluble in an aqueous phase.

(iii) adding two food-grade co-solvents, e.g., propylene glycol or glycerol (water soluble) and ethanol (water and oil soluble) in order to increase the solubility of the polar lipid both in the water and oil phases.

Fig. 2 shows typical phase diagrams which are obtained using a polysorbate (e.g. Tween 80) as the polar lipid [23]. Polysorbates are used in food industry especially as an effective emulsification aid. It is obvious that in this system the isotropic microemulsion region is significantly larger than in the classical food microemulsion system shown in Fig. 1. Note that in the presence of enough glycerol and ethanol (Fig. 2c), a 1-phase channel is formed. This means that a Tween 80/(limonene/ETOH: 1:3) 1:1 ratio (by weight) mixture can be infinitely diluted with water/glycerol (3:1) without passing one or more phase boundaries (multiphase region). This type of microemulsion is also denoted as 'U-type microemulsion' [23]. Inspection of Fig. 2 reveals that addition of both glycerol and ethanol is needed

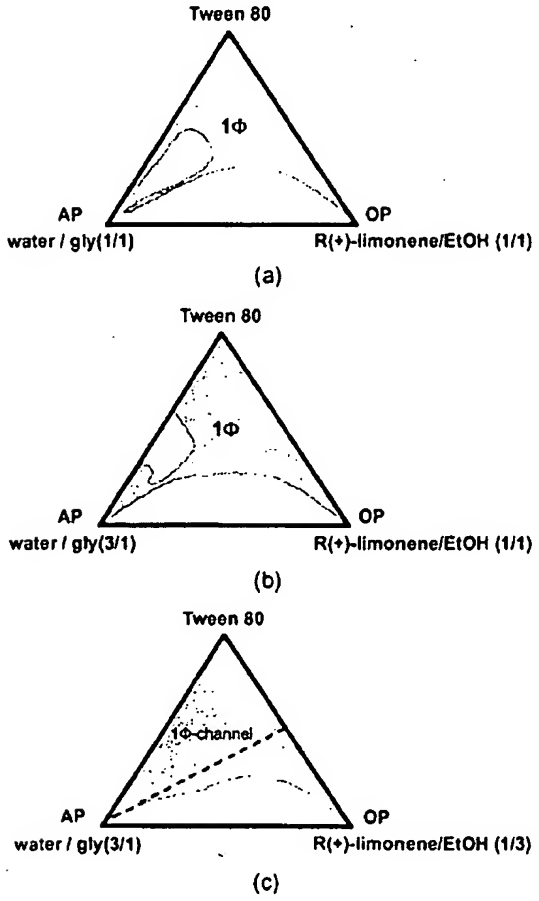

Fig. 2. Pseudo-ternary phase diagrams at 25 °C of the Tween 80/water/glycerol/R(+)-limonene/ethanol mixture. Water/glycerol and R(+)-limonene/ethanol were used in fixed weight ratios. AP: aqueous phase; OP: organic phase: (a)–(c) have different water/glycerol and R(+)-limonene/ethanol ratios. The grey areas represent single-phase microemulsion regions; adapted from [23].

to form the 1Φ-channel. DeCampo et al. investigated the structural evolution of the U-type microemulsion (composition as shown in Fig. 2c) by means of SANS (small angle neutron scattering), dynamic light scattering, conductivity, and viscosity measurements when going from the oil-free micellar Tween–glycerol–water region to the water-free Tween–ethanol–limonene region [24]. In the oil-free region slightly elongated Tween 80 micelles are formed which have a maximal dimension of approximately 13 nm. Adding the limonene/ethanol oil induces swelling of the micelles. The ethanol is distributed in a quite complex way in the system. It could be shown that it was not only partly present in the aqueous and the oil phases, but was also significantly distributed into the interface acting as a co-surfactant [24]. The presence of ethanol causes a lower aggregation number of Tween 80 molecules in the micelles and, thus, makes them shrink in size. The addition of glycerol to Tween 80 micelles increases both the aggregation number and packing density of the surfactant resulting in a slight increase in micellar size. This is due to the fact that glycerol competes with the ethylene oxide groups of Tween 80 molecules for the hydrating water and, as a consequence, makes the surfactant less hydrated leading to a reduced effective volume.

In conclusion, the structural analysis of the 1Φ-channel leads to the assumption that the U-type microemulsion undergoes a continuous structural change from an L_1 (o/w microemulsion) into an L_2 (w/o microemulsion) phase via the formation of a bicontinuous microemulsion structure. The bicontinuous microemulsion phase structure cannot be deduced directly from SANS but from self-diffusion NMR measurements [25–27]. The potential applications of such microemulsion systems are discussed in Section 5.

4. Lyotropic liquid crystalline mesophases obtained from polar food lipids

4.1. General phase behavior of polar lipids

Polar lipids, such as phospholipids or monoglycerides, form lyotropic liquid crystalline self-assembly structures when added to water. These are highly organized structures possessing long-range order, although they are highly disordered on the molecular length scale [8]. Fig. 3 shows a hypothetical lipid/water binary phase diagram which illustrates the different possible mesophases that can form when varying the water content in the system [28]. At high water content normal micelles (o/w microemulsion; L_1 phase) are created. Decreasing the water content, a normal hexagonal (H_1), a lamellar liquid crystalline (L_α), a reversed hexagonal (H_2) and a reversed micellar (w/o microemulsion; L_2) phase appear. In between these phases, different cubic liquid crystalline phases are expected to form: the micellar cubic (I_1), the normal bicontinuous cubic (V_1), the reversed bicontinuous cubic (V_2) and the reversed micellar cubic (I_2). The L_α phase, which has both a zero mean and Gaussian curvature, splits the diagram into two parts: on the higher water content side, the normal ('oil–water') type self-assembly structures are formed (type 1) having a positive mean curvature (the lipids are curved towards the lipophilic chain region), whereas on the lower water

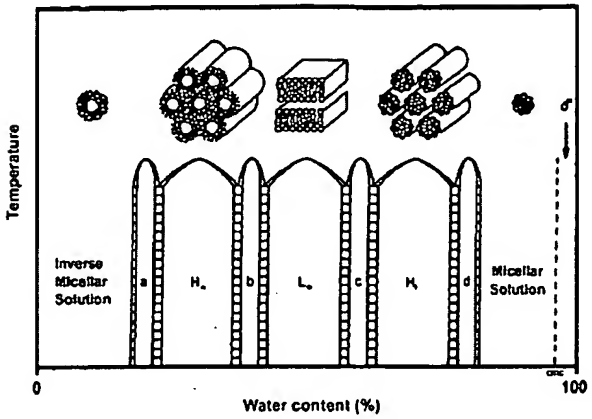

Fig. 3. Hypothetical lipid/water binary phase diagram as a function of the water content; (a) reversed micellar cubic phase (I_2); H_{II}: reversed hexagonal phase; (b) reversed bicontinuous cubic phase (V_2); L_α: lamellar phase; (c) normal bicontinuous cubic phase; H_I: normal hexagonal phase; (d) normal micellar cubic phase (I_1); reprinted with permission from [28].

content side the reversed type ('water-in-oil') structures (type 2) having a negative mean curvature (layer curved towards the water), are formed.

4.2. Phase behavior of monoglycerides

Monoglycerides are the most abundant polar lipid in Food Industry (see Section 1). The self-assembly properties of this class of lipids were started to be investigated in 1960s by several research groups: Lutton [29], Larsson [30] and Luzzati [31] published the first phase diagrams of different monoglyceride–water systems. The samples were examined by observation between crossed polarizers, and the 'consistency' (viscosity) was checked by simply tilting the sample tubes. Later, other more sophisticated methods, such as X-ray diffraction [32,33], DSC (differential scanning calorimetry) [34,35], Electron Microscopy [36] or ^{13}C-NMR [37] were used in combination with visual observations. Especially X-ray diffraction allowed to identify unambiguously the different phases. In particular, it showed that in the monoolein–water system two different reversed bicontinuous cubic phases exist, namely that of symmetry Ia3d,(the underlying minimal surface is gyroid C_g) and *Pn3m* (diamond C_D).

Qualitatively, all monoglycerides show the same sequence of phases as a function of water content and temperature. However, the observed sequence of phases is not totally in accordance with the sequence described in Fig. 3: the reversed bicontinuous cubic phase occurs at a higher water content than the lamellar phase, even though it is classified as a reversed phase. In general, the V_2 is expected to occur at a lower water content than the lamellar phase (Fig. 3).

4.2.1. Phase behavior of monolinolein

Fig. 4 shows the binary phase diagram of the monolinolein (MLO; $C_{18:2}$)–water mixture as an example of a monoglyceride–water system [38]. The MLO (emulsifier TS-PH 039) was supplied by Danisco A/S, Denmark. It is a distilled monoglyceride which is 94% pure monoglyceride. The fatty acid chains are 92% linoleic ($C_{18:2}$), 7% oleic ($C_{18:1}$) and about 1% saturated. Its phase behavior is very similar to that of the monoolein–water system which is the most frequently studied monoglyceride–water mixture [39]. The most striking phase formed in these systems is the reversed bicontinuous cubic phase (V_2), which is stable at room temperature. In the MLO–water system it is formed between 10% and 32% water at 25 °C (Fig. 4). The V_2 phase has extraordinary physical properties: a high internal surface area, a high viscosity (which is the most viscous phase among the existing mesophases) and a viscoelastic rheological behavior [40]. From an application point of view (see below), monoglyceride based cubic phases are safe and not very costly.

The high viscosity of the bicontinuous cubic phase makes preparation of this phase a non-trivial task. Therefore, simple vortexing is not efficient enough and it would take too much time for the water to be distributed homogeneously throughout the phase. Heating helps to make the sample less viscous, especially when it is possible to reach the fluid L_2 phase (see Fig. 4). Therefore, one of the easiest ways to make a bicontinuous cubic phase is to heat the monoglyceride up to the temperature where the L_2 phase is formed. The subsequent addition of hot water while vortexing or homogenisation allows to create quite easily a homogeneous mixture which transforms upon cooling spontaneously into the V_2 phase. Note that it is more tedious to make the cubic phase with the monoolein than with the monolinolein, since the monoolein cubic phase is already formed below 90 °C, whereas the monolinolein cubic phase forms only below ca. 50 °C. This makes the latter system less sensitive to temperature fluctuations and allows to homogenise the monoglyceride–water mixture more easily, since the L_2 (or H_2) phase is stable to lower temperatures.

4.2.2. Phase behavior of commercially available monoglyceride mixtures

The MLO–water phase diagram is very similar to the phase diagram of the commercially available monoglycerides, such as

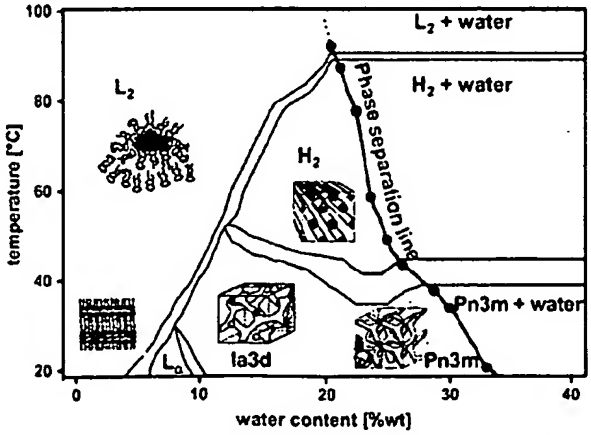

Fig. 4. Binary monolinolein/water phase diagram as a function of temperature, as determined by SAXS. The phase separation line separates the single-phase and the excess water region; reprinted with permission from [38].

the DIMODAN LS or DIMODAN U from Danisco (made from sunflower oil). The fatty acid chain composition of the DIMODAN U monoglyceride is less pure (67% $C_{18:2}$; 21% $C_{18:1}$; ca. 11% saturated chains) than that of the MLO monoglyceride preparation. Differences are mainly observed in the temperature stability of the L_α and V_2 phases. The melting temperature for both phases is lower in the MLO–water than in the DIMODAN U–water system. This is certainly linked to the fact that the MLO consists of more $C_{18:2}$ chains (which is more unsaturated) than the DIMODAN U. However, the transition temperature between the H_2 and L_2 phases is very similar in both systems.

When studying the phase behavior of commercially available samples, it is important to take also into account the possibility of metastable phase behavior (denoted also as metastability) [39]. Metastable phase behavior is mainly encountered at temperatures below 20–30 °C, i.e., where undercooling of the liquid crystal phases occurs. Clogston et al. [41] investigated this phenomenon in a commercially available monoolein–water system. When samples were slowly cooled down from 20 °C, the bicontinuous cubic phase was found to persist as a pure phase down to 0 °C. These are, however, only metastable conditions, since at equilibrium conditions, L_c crystals appear which are in equilibrium with the cubic phase (*Pn3m*) or water/ice (at temperatures below 0–10 °C). Similar metastable effects are also observed in the pure monoolein–water system [39]. This indicates that metastability can come from both the crystallization behavior of the monoolein itself and from the behavior of the saturated monoglycerides present in the commercial sample.

4.3. Nanostructured particles from reversed monoglyceride self-assembly phases

4.3.1. Monoglyceride–water dispersions

In order to enhance the application areas for lipid self-assembly structures people have tried to disperse bulk mesophases into water. For instance, lamellar phases are easily dispersed into water in the form of well-known vesicle or liposome structures [42]. Dispersion of reversed liquid crystalline mesophases, however, is more complex. They show only a limited stability in water and quickly phase-separate after dispersion. Inherent to these systems is the problem of lipophilicity of the surfaces formed after fragmentation of these mesophases. Since the particle inner structure is bent towards the water phase (which has a negative natural curvature, see Fig. 4), the lipophilic domains are exposed to the continuous water phase, which is energetically unfavourable. Therefore, in order to stabilize these lipophilic particles, an extra stabilizer has to be added [43,44].

The first examples of a fragmented reversed polar lipid phase were described by Patton and Carrey [45] and Lindström et al. [46] some 25 years ago. Most work relevant to food, so far, was on the dispersion of reversed bicontinuous cubic phases, made of monoolein (GMO). Landh [47] found that amphiphilic block copolymers provided exceptional stabilization of reversed bicontinuous cubic phase dispersions, most probably through steric stabilization. The Poloxamer 403 (from BASF), also denoted as Pluronics F-127, is by far the most studied block copolymer in this context. The dispersed bicontinuous cubic particles were denoted as 'Cubosomes' and the dispersed reversed hexagonal particles as 'Hexosomes' [43,44].

In Fig. 5 Cryo-TEM images of a cubosome and a hexosome are shown [38]. Note that the cubosomes frequently coexist with attached vesicular structures. Heating up the same dispersion to 55 °C transforms the cubosome into a hexosome (Fig. 5b). Hexosomes can either show an internal hexagonal pattern with outer hexagonal morphology, or curved striations similar to those shown in Fig. 5b. The striations are hexagonally packed tubes, which are bent together.

Recently, Sagalowicz et al. [48] showed that Cryo-Transmission Electron Microscopy (Cryo-TEM) in combination with Fast Fourier Transform (FFT) and tilting experiments was an effective alternative to SAXS (see below) to obtain information on the crystallographic structure and the space group of particles having reversed bicontinuous cubic and hexagonal structures. A major advantage of Cryo-TEM is the possibility to analyze single particles. This allows identification of particles present at very low concentrations and the coexistence of particles having different internal self-assembly structures. Using this technique one can visualize that bicontinuous cubic phase particles with

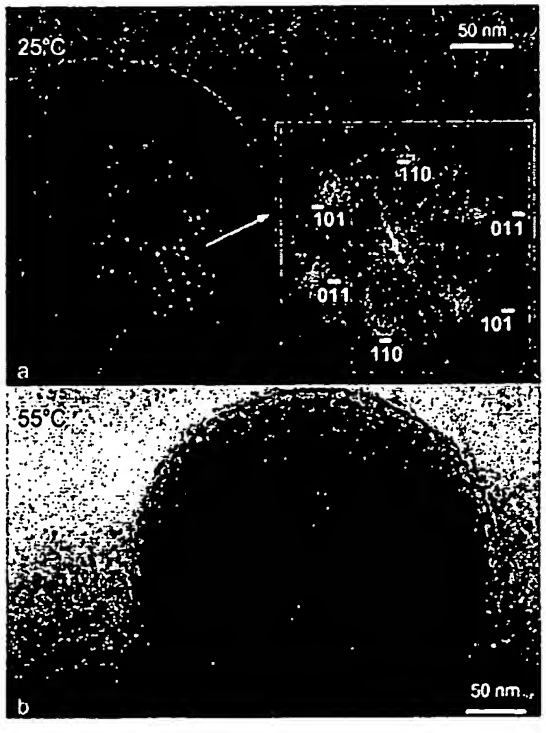

Fig. 5. Cryo-TEM images of a dispersion consisting of 4.63 wt.% MLO, 0.38 wt.% F127, and 95.0 wt.% water; adapted from [38]. (a) Cubosome at 25 °C; particle together with some vesicular structure is observed. The Fourier transform of the internal structure of the particle (inset) shows a hexagonal arrangement with interplanar distances *d* of about 6 nm. The internal structure is observed along the [111] axis, and the crystallographic planes observed are of {110} type. This is compatible with a cubic structure of *Pn3m* symmetry with a lattice parameter *a* of 8.5 nm. (b) Hexosome at 55 °C; particle exhibits curved striations.

different space groups can coexist within the same dispersion [48].

Using the method of Gustafsson et al. [43], it is indeed possible to disperse the cubic and hexagonal phases without losing the structural features of the bulk phase inside the particles after dispersion. Fig. 6 shows the corresponding Small Angle X-ray scattering (SAXS) curves obtained from the non-dispersed reversed bicontinuous cubic (*Pn3m*), reversed hexagonal (H_2) and the reversed microemulsion (L_2) phases together with their corresponding dispersion [38]. The resemblance of the respective curves is remarkable. The peak positions are practically identical. The peaks of the dispersions are slightly broadened and show an upturn at the lowest q values, both of which are related to the limited size of the dispersed particles (about 200 m). This means that neither the confined geometry imposed on these phases (limited size of the particles) nor the polymer (at the applied concentration) used to stabilize the dispersion (F127) significantly affect the internal nanostructure in the particles. The internal particle phase is in equilibrium with the surrounding excess water phase. Thus the particles expel water upon heating (transition from V_2 to H_2 to L_2, see phase diagram in Fig. 4) and take up water when cooling down again [38].

Fig. 6 shows another remarkable feature: this concerns the fact that in this system the w/o microemulsion phase (formed at high temperatures) can be dispersed without losing the characteristics of the L_2 structure in the particle [38]. The L_2 phase particles consist of hydrophilic domains having no long-range order. Such a dispersion can also be denoted as 'emulsified w/o microemulsion', i.e., as an o/w emulsion in which the oil droplets have the structure of a w/o microemulsion. It should be

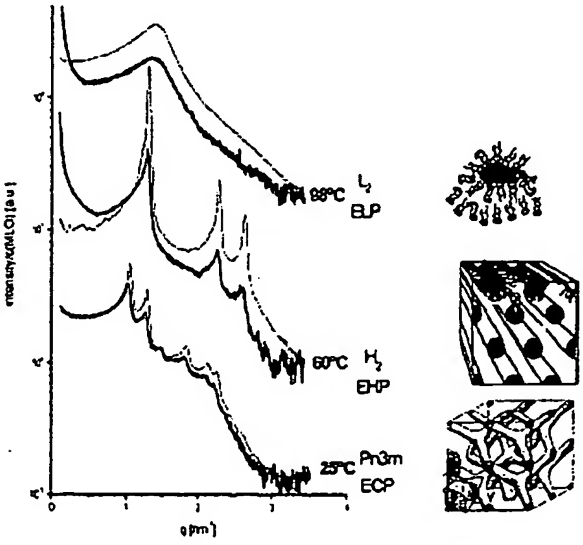

Fig. 6. Comparison of the SAXS scattering curves obtained from the formulated dispersions (thick line) and the respective bulk phase in the presence of 40% excess water (thin line) at different temperatures. Intensities were normalized by the respective MLO concentration: ECP: emulsified cubic phase; EHP: emulsified hexagonal phase; ELP: emulsified L_2 phase; with permission from [38].

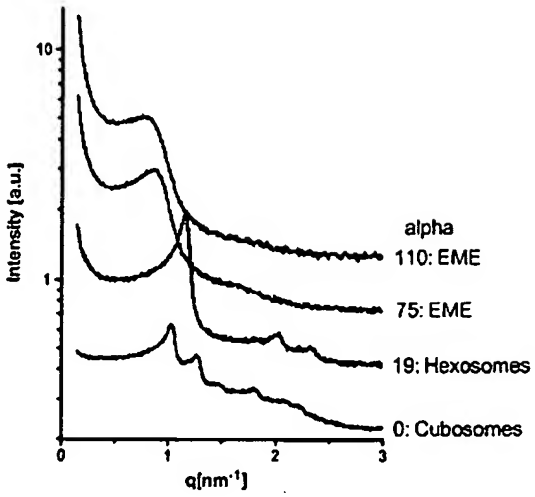

Fig. 7. Effect of adding tetradecane on the SAXS scattering curves of MLO based dispersions at 25 °C; α = oil/MLO weight ratio × 100; Pluronic F127 was used as dispersing agent; dispersion composition: 4.6% lipidic phase (MLO+ tetradecane); 0.4% F127; with permission from [53].

noted that emulsification of w/o microemulsions made out of classic synthetic surfactant–hydrocarbon mixtures with water is not a trivial task. Homogenisation of such w/o microemulsions–water systems (Winsor II systems) at constant temperature, generally, leads rather to w/o emulsions instead of o/w emulsions [49].

4.3.2. Influence of additives on monoglyceride–water dispersion properties

With a view to use cubosome or hexosome dispersions for the delivery of hydrophilic and lipophilic molecules (see Section 5), some work has been published on the effect of such additives ('guest' molecules) on the binary lipid–water system. The main result of these studies is that all additive molecules significantly influence the phase behavior of the binary monoglyceride–water system. After solubilization of a certain 'critical' amount of additive, a phase transition is induced. Each additive affects the interfacial curvature of the lipid bilayer in a different way. Lipophilic additives, such as oleic acid [50,51], triglycerides [43,52] or tetradecane [53] induce a transition from a reversed bicontinuous cubic to reversed hexagonal phase, i.e., the mean interfacial curvature becomes more negative. More hydrophilic additives, such as the DGMO (diglycerol monooleate) [54] induces the transition from the reversed bicontinuous cubic to the lamellar, i.e., the mean interfacial curvature changes towards zero. However, addition of hydrophilic molecules, such as polysaccharides, can also induce a transition towards more negatively curved phases. Mezzenga et al., recently, showed that the presence of hydrophilic mono-, oligo-, and polysaccharides in the bicontinuous cubic mesophase resulted in a decrease of the cubic-to-hexagonal transition temperature [55]. The authors state that in the presence of hydrophilic compounds with a strong hydrogen bond acceptor the number of water molecules hydrating the monoglyceride polar head is reduced. Thus, provided

that the polysaccharide is small enough to fit within the water channels of the cubic phase, the change in the critical packing parameter is proportional to the number of hydrophilic groups (carbohydrate concentration) dissolved in the water phase.

Yaghmur et al. studied in more details the effect of adding tetradecane (TC), i.e., a lipophilic additive, to a monolinolein–water bicontinuous cubic phase dispersion (cubosomes) by means of SAXS and Cryo-TEM [53]. As shown in Fig. 7, addition of tetradecane (i.e., increasing the TC/MLO weight ratio at constant total lipid content; $\alpha = \text{TC/MLO} \times 100$) transforms the bicontinuous cubic $Pn3m$ structure inside the dispersed particles at room temperature into the H_2 (at $\alpha = 19$) and the L_2 (at $\alpha \geq 75$) structures. Addition of tetradecane induces the same transitions when using bulk phases. Note that at $\alpha = 75$ and 119 an 'emulsified microemulsion' is observed. Its structural features are qualitatively the same as observed already in the binary monolinolein–water dispersion at 98 °C (see Fig. 6). This indicates that, principally, it is possible, again with the help of a stabilizer, to form emulsified microemulsions also at room temperature without losing the internal w/o microemulsion self-assembly structure of the dispersed lipid particles.

5. Potential applications of polar lipid self-assembly in foods

In the following section we will sketch some potential applications, which have been described in the literature so far. The idea is not to provide an exhaustive review, but rather to present some highlights. Especially we will discuss the potential applications in relation to the underlying self-assembly properties. We will concentrate on three main application areas: on (i) solubilization, (ii) controlled release, and (iii) chemical reactivity. Certainly, more application areas are already under investigation or will emerge in the future.

5.1. Solubilization of poorly water soluble ingredients

One of the most interesting applications for self-assembly structures, such as microemulsions or lyotropic liquid crystalline phases, in food is to facilitate the addition of food ingredients. In particular, flavors, preservatives or nutrients that are poorly soluble in water can be incorporated in water-based foods by solubilizing the component(s) within polar lipid self-assembly structures. Both microemulsions (or micelles) [11,12,15,56] and liquid crystalline mesophases [50,57–61] have been investigated with respect to their solubilization potential.

Recently, Garti et al. [56,62] described in more details the solubilization capacity of several lipophilic nutrient molecules, such as lutein, phytosterol or lycopene into U-type microemulsions (described in Section 3.3). All three neutraceuticals are insoluble in water and only poorly soluble in oil. It should be mentioned that food systems usually require relatively high solubilization (loading) capacities of the guest molecules compared to drugs used in pharmaceutical applications, since (i) the required 'dosage' of the nutrients in foods is typically higher than for drugs, and (ii) the amount of emulsifier phase added to the final product for delivering functional molecules needs to be as low as possible due to regulatory issues.

The U-type microemulsion system used is based on Tween 80 (or 60) (as surfactant), R(+)-limonene (as oil), glycerol (or propylene glycol) and ethanol as co-solvents (see Section 3.3.). One strategy to use these microemulsions as a delivery system for lipophilic components is to prepare first an oil concentrate which contains the surfactant, oil and co-solvent giving rise to the formation of reverse micelles. The reverse micelles enhance the 'solubility' of lipophilic components in the oil phase. Then the loaded oil concentrate is added to a water-based food product, for instance, a beverage. The advantage of using U-type microemulsion formulations is that mixing the oil concentrate with a water-based product takes place without significant phase separation [62]. Therefore, it allows fortifying products, such as clear beverages, with lipophilic neutraceuticals without rendering the product turbid or inhomogeneous. A requirement for the successful use of this fortification method is that the added microemulsion self-assembly structures do not interact with the other components present in the product 'damaging' the structure of the microemulsion (change in phase behavior).

Recently, Garti et al. [62] quantified the 'oil solubilization efficiency' of phytosterols, lutein and lycopene into Tween 80/R (+)-limonene/ethanol reverse micelles. The study showed that the presence of Tween micelles increased solubility of the neutraceuticals by factors of 15, 11, and 5, respectively, compared to the solubility observed in the limonene–ethanol mixture alone. The results show that the increased oil solubility of the phytosterols is mainly due to the fact that the phytosterol molecules are solubilized into the Tween-based interfacial region of the reverse micelles. Lutein is accommodated less well and its total solubilization is lower. Lycopene, on the contrary, is difficult to accommodate in the reverse micellar solution. It is solubilized to a much lower extent than the other neutraceuticals. This indicates that phytosterols and lutein show a reasonable surface activity and easily penetrate the micellar interface, while lycopene seems to be much less surface active.

Adding the loaded oil concentrate to the aqueous phase induces the reverse micelles to swell forming a w/o microemulsion (change of interface curvature). Dilution with the aqueous phase reduces phytosterol solubilization into the interface considerably [62]. Lycopene solubilization, however, is influenced to a much lesser extent since it is not preferentially solubilized into the interface. A change in the interface curvature, therefore, has only little effect. After addition of 50% aqueous phase the microemulsion has a bicontinuous structure. While the phytosterol solubilization into the interface drops further with aqueous phase addition, lutein solubilization into the interface increases [62]. In the presence of 80–90% aqueous phase an o/w microemulsion is formed and the interface is convex towards the oil. A careful analysis of the solubilization data in this region reveals that lycopene solubilization into the o/w interface is preferable to solubilization into the w/o interface. The opposite was found for phytosterol and lutein.

In conclusion, one can say that the surfactant interface layer as formed in microemulsions is an important site for guest molecules, like neutraceuticals, preservatives, or flavors. It can be used to significantly increase the dissolution of water-insoluble and poorly oil-soluble components in a lipidic phase. This phase

can be added as a concentrate to any water-based food product. In principal, this concept can be applied to fortify any aqueous-based food product unless the U-type microemulsion delivery system is physically 'decomposed'. The main parameters to control are the nature (curvature, composition) of the microemulsion interface and the interaction of the microemulsion droplets with other food components.

5.2. Controlled release

Controlled release is a process that can be used to increase the effectiveness of ingredients, such as flavors, nutrients, etc., in food products. Its initial roots are in the pharmaceutical industry that aims at developing injectable drug formulations that give a sustained release and avoid the supply of high transient drug levels. The most common way to control the release of active compounds is by microencapsulation, i.e., by creating a microcapsule in which the active ingredient containing core matrix is surrounded by a wall or barrier [71]. The release rate from such a reservoir-type system depends on the thickness, the area of the capsule surface, and the permeability of the barrier. Principally, it is dependent on the diffusion of the active ingredient within the core reservoir and through the barrier [71]. The extent of release (how much of the active component can maximally diffuse out of the capsule) is defined by the partition coefficient between the microcapsule and its environment.

Vauthey et al. [63] compared the release pattern (pseudo-equilibrium situation) of a mixture of 8 aroma compounds from different structured and non-structured phases. The main question was whether the aroma release properties were influenced by the formation of self-assembly structures, i.e., the interface. Seven metal oxide semiconductor gas sensors ('Electronic Nose') were used to monitor the aroma composition in the respective headspace as a function of time. Fig. 8 shows the results of the statistical treatment of the release data using the principal components analysis (PCA analysis). Clearly the four used matrices create different aroma profiles in the headspace measured after 40 min of release. Since about 98% of the information is contained in the first principal component, the 2-D plot given in Fig. 8 can practically be reduced to a 1-D graph (x-axis), which is proportional to the concentration of measured headspace volatiles. On the left-hand side of the graph, a cluster comprising volatiles released from pure sunflower oil and monoglyceride is observed. This cluster exhibits the lowest concentration of molecules in the gaseous phase, i.e., the partitioning into the headspace is lowest. On the other hand, the volatiles entrapped in the aqueous phase are released in the highest amounts (the cluster on the right-hand side in Fig. 8) indicating the highest partitioning into the headspace. Whereas the w/o emulsion and the bicontinuous cubic phase (made from unsaturated monoglycerides) have the same water content (20 wt.%) the headspace concentration of the volatiles released from the cubic phase is different than the headspace concentration released from the w/o emulsion. This result suggests that the structure of the matrix significantly determines the partitioning of the volatiles. Whereas the water in the w/o emulsion, which is stabilized by 1% of the emulsifier PGPR (polyglycerol polyricinoleate), is distributed in micrometer-sized droplets, the water in the cubic phase is distributed in nano-sized channels stabilized by a huge interfacial area (estimated to be 400 m^2/g cubic phase). As a result, it is very likely that, in comparison to the emulsion, a certain amount of volatile components will be solubilized into the internal interface of the bicontinuous cubic phase and, therefore, in contact with the water domains in the cubic phase. This is expected to lead to a larger partition coefficient, and, therefore, to a higher equilibrium volatile headspace concentration.

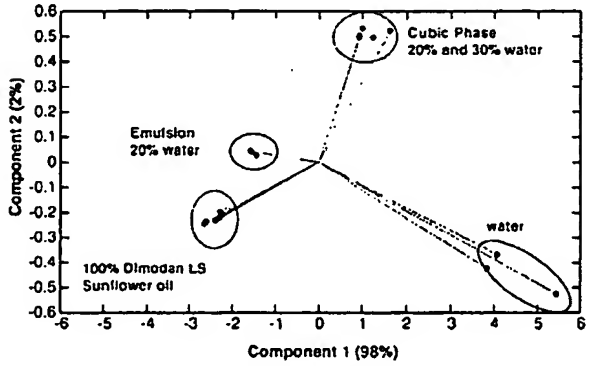

Fig. 8. Principal component analysis of E-nose headspace data obtained after the release of a model aroma mixture (for more details see [63]) from different matrices, i.e., from 100% Dimodan LS or sunflower oil, w/o emulsion containing 20% water, bicontinuous cubic phase containing 20% or 30% water, and water; headspace data are based on the response of an array of seven non-specific gas sensors after 40 min of release; adapted from [63].

Recently, Boyd discussed the release of some lipophilic drugs from cubosomes and the corresponding bulk bicontinuous cubic phase into a water solution. He showed that the release from submicrometer sized cubosomes was over 200,000 times greater than the release from the corresponding bulk bicontinuous cubic phase [64]. This is linked to the fact that when the cubic phase is dispersed as particles a much larger (cubic phase-external water) interface is formed than when the cubic phase is not dispersed but is only in contact as a whole with the external water phase. Note that the release of the drug per unit surface area is constant. Moreover, the release from the bulk cubic phase has been shown to follow the Higuchi diffusion controlled kinetics [64], where the amount of drug released per unit area of matrix shows a linear dependence with the square root of time and the diffusion coefficient. This result indicates that cubosomes, such as emulsion droplets, usually show a 'burst-type release' that will not provide sustained release profiles for lipophilic drugs. The use of functionalised phospholipids as a possible means of retaining the drugs inside the cubosomes has been described recently by Lynch et al. [65]. More systematic research, however, is needed to better understand the release pattern of more hydrophilic and/or amphiphilic molecules.

In conclusion, it should be mentioned that the location where the guest molecules are present in the self-assembly system (the

oil, interface or water phase) will determine how much and how fast a certain 'guest molecule' will be released from its 'host', i.e., delivery system. Solubilization into the interfacial area influences mainly the partitioning behavior of the guest molecules between the structured medium and the environment determining whether a 'burst-type' or sustained release is observed.

5.3. Self-assembly structures as reaction media

Taste or flavor release properties of a food product during consumption are one of the major criteria defining the quality of a product. In foods, flavors are generally created via the Maillard reaction, i.e., the thermally induced reaction between a reducing sugar and an amino acid. Self-assembly systems are attractive 'nanoreactors' for such reactions, since solubilization phenomena are expected to influence the reaction pathway and/or yield. Vauthey et al. [66] studied a model Maillard reaction, namely the reaction between L-cysteine and furfural in two different self-assembly structures, i.e., an L_2 isotropic phase and a bicontinuous cubic phase at 100 °C. The self-assembly structures were made with monoglycerides (a saturated monoglyceride was used to make the cubic phase and an unsaturated monoglyceride was used to make the L_2 phase at 100 °C). The water content in both systems was the same, namely 20%. Of particular interest was to follow the generation of 2-furfurylthiol (FFT). This compound is a potent odorant with unique sensory properties. It belongs to the key impact compounds of heated cysteine–ribose (or furfural) mixtures [67]. It was shown that the reaction yield of the FFT in the self-assembly structures was significantly increased compared to the reaction in the phosphate buffer at pH 5 (see Table 4). This rate enhancement can be due to (i) a local increase of the reactants concentration at the interface (close proximity of the reactants), or (ii) the specific environment at the surfactant–water interface that may lower the activation energy of the reaction (restrained movement of the reactants within the interface (cage effect)), or (iii) the partitioning of lipophilic reaction products between the hydrophilic and lipophilic domains in the self-assembly structures (which lowers the actual concentrations of the reactant products in the water). Interestingly enough the experiments showed also that not only the yield of the FFT generation was enhanced, but also two new sulfur compounds were created (Table 4, [66]) which were not observed in the respective water-based reaction.

Table 4
Amounts of volatile reaction products generated from furfural and cysteine solubilized in different matrices (adapted from [66])

Matrix	Amount (μg)[a]		
	2-Furfurylthiol	2-(2-Furyl)-thiazolidine	*N*-(2-mercaptovinyl)-2-(2-furyl)-thiazolidine
Water	1.8	n.d.[b]	n.d.[b]
Microemulsion	8.7	42	11.7
Cubic phase	11.6	240	123

[a] Mean values of duplicates (S.D. < 20%).
[b] Not detected.

Garti et al. [68,69] found the same reaction characteristics (enhanced formation of FFT; generation of the same two new sulfur components) when the reaction was performed in sucrose stearate/dodecane/butanol w/o microemulsions at 60 °C. Moreover, they studied the reaction as a function of the microemulsion water content. It was found that the reaction rate decreased significantly with increasing water content in the w/o microemulsion. This effect is linked to the partitioning of the butanol between the water core, the water–oil interface and the oil. Because the furfural is completely soluble in the butanol but is only slightly soluble in the oil, and insoluble in the other microemulsion components, it mainly partitions together with the butanol between the three locations. The cysteine partitions between the water and the interface as it is hydrophilic. Since cysteine in not soluble in the oil phase, and furfural is not soluble in the aqueous phase, the reaction is thought to take place favourably at the interface. At low water content the quantities of butanol (and therefore also of furfural) and cysteine at the interface are high giving rise to a high reaction yield. Increasing the water content in the system decreases the butanol (and also the furfural) and cysteine contents present at the interface leading to a slower reaction rate.

The same Maillard reaction was also carried out in U-type microemulsions that allow studying the influence of the microemulsion interface curvature on the reaction yield [70,72]. The curvature can be continuously varied by varying the water content in the microemulsion system (see Section 3.3). One main result of this study was the observation that the reaction rate was significantly higher in the o/w than in the w/o microemulsion region. Moreover, the ratio of the two newly formed sulfur compounds is very different in the two types of microemulsions. One possible explanation for this is related to the different partitioning behavior of the reactants at the interface, especially to the partitioning of the furfural. The more water is in the system, the higher is the furfural concentration at the interface, and, thus, the higher is the initial reaction rate. Another possible explanation is linked to the fact that cysteine is thermally unstable when heated in the presence of water generating reactive intermediates which can further react with the furfural.

In conclusion, it can be stated that the interface present in self-assembly structures containing liquids can be used to tune and modulate molecular reactivity. This application is, however, still in its infancy, and more systematic work has to be carried out in order to be able in the future to predict reaction yield and pathway as a function of the interfacial properties of the self-assembly structures, such as curvature or composition.

6. Concluding remarks

Polar food lipids when placed into water are able to form a great variety of self-assembly structures. The possibility to solubilize insoluble active ingredients into self-assembly structures can be used for the formulation of new and improved functional food products. Controlling the release or reactivity of functional molecules using self-assembly structures can be applied to make tastier products. A future challenge for the food industry will be to deliver nutrition and health-related food

products adapted to the individual needs of consumers. One way to meet this challenge is to use molecular self-assembly properties of polar lipids for the in-situ generation of specific structures providing an intrinsic delivery function.

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(X) RELATED PROCEEDINGS APPENDIX

None.